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Ruthenium(III) S-methylisothiosemicarbazone Schiff base complexes bearing PPh_3/$AsPh_3$ coligand: Synthesis, structure and biological investigations, including antioxidant, DNA and protein interaction, and *in vitro* anticancer activities

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ABSTRACT

New Ru(III) isothiosemicarbazone complexes [$RuCl(EPh_3)L^{1-4}$] (E = P or As) were obtained from the reactions between [$RuCl_3(EPh_3)_3$] and bis(salicylaldehyde)-S-methylisothiosemicarbazone (H_2L^{1-3})/ bis(2-hydroxy-naphthaldehyde)-S-methylisothiosemicarbazone (H_2L^4) ligands. The new complexes were characterized by using elemental analyses and various spectral (UV–Vis, IR, ^1H NMR, FAB-Mass and EPR) methods. The redox properties of the complexes were studied by using cyclic voltammetric method. The new complexes were subjected to various biological investigations such as antioxidant assays involving DPPH radical, hydroxyl radical, nitric oxide radical and hydrogen peroxide, DNA/protein interaction studies and *in vitro* cytotoxic studies against human breast cancer cell line (MCF-7). New complexes showed excellent free radicals scavenging ability and could bind with DNA via intercalation. Protein binding studies using fluorescence spectroscopy showed that the new complexes could bind strongly with bovine serum albumin (BSA). Photo cleavage experiments using DNA of *E-coli* bacterium exhibited the DNA cleavage ability of the complexes. Further, the *in vitro* anticancer activity studies on the new complexes against MCF-7 cell line exhibited the ability of Ru(III) isothiosemicarbazone complexes to suppress the development of malignant neoplastic disease cells.

1. Introduction

The alchemy of metal complexes offers enormous opportunities for the design and development of compounds with excellent bio-activities due to the assortment of available metals and the ability to tune the reactivity and structure of the metal complexes by their ligand sphere [1,2]. The biological activities of the metal complexes are widely influenced by the nature of metal ions, its oxidation state, the type and nature of bound ligands and isomers [3–5]. Hence, the understanding of the factors affecting the biological activity of metal complexes should enable the design of metal complexes with specific medicinal properties and also to overcome the disadvantages of available medicines. For example, cisplatin, a platinum(II) diamine complex used in 70% of cancer treatment has some drawbacks like toxic side-effects and lack of activity (drug resistance) against several types of cancer which are problems need to be overcome [6]. So, the search for anticancer activity amongst complexes of other metals has received much interest. Currently, ruthenium complexes have been found as an attractive alternate for platinum due to several favorable properties suited to rational anticancer drug design and biological applications [7–9] such as their bio-compatible ligands exchange kinetics similar to platinum, a higher coordination number and lower toxicity toward healthy tissues than their platinum counter parts [10,11].

Experiments showed that the DNA interaction studies of small molecules can be used to design more effective anticancer drugs [12,13]. Considerable interest has been generated in DNA binding and DNA cleavage by metal complexes with redox and photoactive nature, in order to explore the sequence specificities of DNA binding using a variety of intercalating ligands [14–16]. In general, small molecules bind to DNA in non-covalent modes such as intercalation, groove-binding, and external electrostatic binding [17,18] and the intercalation ability of metal complexes correlates

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to coordination geometry, metal ion type, its valence and the ligand donor type [19,20]. The intercalation binding of metal complexes with DNA could induce cellular degradation which is indispensable for the anticancer application of metal complexes [21]. Likewise, knowledge of interaction mechanisms between the drugs and plasma proteins is very important to understand the pharmacodynamics and pharmacokinetics of a drug [22]. Serum albumin is the most abundant soluble protein in animals, including the human circulatory system that is in-charge for the transport, distribution and deposition of a sort of endogenous and exogenous substances in the body [23]. This protein possesses a high homology and a similarity to human serum albumin (HSA) in sequence and conformation [24] and is always chosen as a model of general drug–protein interactions. Further, the uncontrolled production of free radicals or reactive oxygen species such as $OH^{\cdot}$ and hydrogen peroxide causes DNA damages in human and results several diseases including cancer [25,26]. Various ruthenium complexes were reported for their free radical scavenging ability against different harmful free radicals [27–30].

Thiosemicarbazones, their derivatives, as well as their transition metal complexes have aroused considerable interest in the areas of chemistry and biology. These compounds present a wide variety of biological activities such as antitumor [31–35], fungicidal [36,37], bactericidal [38] or antiviral [33] and so on. The thiosemicarbazide fragment =N(1)—N(2)H—C(3)=S—N(4)—NH_2 is a bifunctional chelating agent, and can be coordinated in a bidentate mode to give five-membered metal rings through the N(1) and S atoms (mode 1) or the N(1) and N(4) atoms (mode 2). The coordination of the thiosemicarbazide fragment of the ligand via mode 1 was found in complexes of both 3*d* transition metals and platinum-group metals, namely, Pt(II) and Pd(II) [39,40]. In some cases where this fragment is attached to a metal through the N(2) and S atoms to create a four-membered metal ring was also reported [41]. It is significant to note that one bond in all known structures is always through the sulfur atom [39–42]. Nevertheless the unusual mode 2 is exceptionally observed in some molybdenum (VI) complexes. Thioalkylated derivatives of this class are rather reactive; participate in interesting metal-promoted reactions, which give rise to new sequences of atom and bond combinations and to unusual coordination modes. Particularly, for S-methylisothiosemicarbazone of salicylaldehyde (H_2L), it was shown that thioalkylation results in both a change in the coordination mode from O,N,S to O,N,N and modification of the acid–base properties of the thiosemicarbazide fragment.

As a part of our continuing research in ruthenium chemistry, here we have synthesized a series of hexacoordinated ruthenium(III) S-methylisothiosemicarbazone complexes containing triphenylphosphine/triphenylarsine as co-ligands and characterized them by using elemental and spectral methods (UV–Vis, IR, FAB-Mass and EPR). The radical scavenging ability ($DPPH^{\cdot}$, $OH^{\cdot}$, $NO^{\cdot}$, H_2O_2), DNA and protein interactions and the anticancer property against the MCF-7 cell line of the new complexes were investigated.

2. Experimental

2.1. Materials and methods

All the reagents used were chemically pure and AR grade. The solvents were purified and dried according to standard procedures [43]. $RuCl_3{\cdot}H_2O$ was purchased from Loba Chemie Pvt. Ltd. The starting complexes $[RuCl_3(PPh_3)_3]$ and $[RuCl_3(AsPh_3)_3]$, S-methyl thiosemicarbazide and S-methylisothiosemicarbazone Schiff bases were prepared according to the literature procedures [44–47].

2.2. Physical measurements

Microanalyses (C, H, N and S) were carried out on Vario EL III Elemental analyzer at SAIF, Cochin, India. The IR spectra of the ligands and their complexes were recorded as KBr pellets on a Nicolet Avatar model FT-IR spectrometer in 4000–400 cm^{-1} range. Electronic spectra of the ligands and their complexes have been recorded in dichloromethane using a Shimadzu UV-1650 PC spectrophotometer at 800–200 nm range. ^{1}H NMR spectra were recorded in Jeol GSX-400 nuclear magnetic resonance spectrometer using TMS as internal reference. Electron paramagnetic resonance spectra (EPR) of the powdered samples were recorded with a JEOL JES-FA200 electron spin resonance spectrometer with X-band frequencies at room temperature using 2,2′-diphenyl-1-picrylhydrazyl radical (DPPH) as internal standard at Pondicherry university, Pondicherry, India. The FAB-MS spectra were recorded by EI technique using the JEOL JMS600H instrument in the HRMS facility NIIST, Thiruvananthapuram. Magnetic moments were measured using an EG and G-PARC vibrating sample magnetometer. Cyclic voltammetric measurements were carried out with a BAS CV-27 electrochemical analyzer in acetonitrile solution using tetrabutyl ammonium perchlorate (TBAP) as supporting electrolyte. Three electrode cell was employed with glassy carbon working electrode, a platinum wire as counter electrode and an Ag/AgCl as a reference electrode. Melting points were recorded on a Technico micro heating table and are uncorrected.

2.3. Preparation of S-methylisothiosemicarbazone ligands

A mixture of thiosemicarbazide (10 mmol) and methyl iodide (10.6 mmol) in absolute ethanol (20 mL) was refluxed for 45 min and allowed to cool at room temperature for 12 h. White crystals of S-methylthiosemicarbazide hydrogen iodide separated which was filtered, washed with ethanol and used for the next step without further purification. Yield: 68%. In the second step, a mixture of S-methylthiosemicarbazide hydrogen iodide (5 mmol) and corresponding aldehyde (10 mmol) in absolute ethanol was neutralized with an aqueous $Na_2CO_3{\cdot}10H_2O$ solution and kept overnight. The pale yellow crystals of the ligands obtained were filtered off and washed with water and finally with ethanol. Yield: 85–90%.

2.3.1. Bis(salicylaldehyde) S-methylisothiosemicarbazone (H_2L^1)

H_2L^1 was prepared from the reaction of S-methylthiosemicarbazide (1.165 g, 5 mmol) with salicylaldehyde (1.221 g, 10 mmol). Yield: 68%. Color: Yellow solid. M.p: 135 °C. Anal. Calc (found). ($C_{16}H_{15}N_3O_2S$): C, 61.32(61.46); H, 4.82(4.71); N, 13.41(13.21); S, 10.23(10.18). IR (KBr, cm^{-1}): 3265(b) ν(O—H^1); 3280 ν(O—H^2); 1617(s) ν(C=N^1); 1635(s) ν(C=N^2); 1377 ν (C—O). UV–Vis (λ_{Max}, nm (ε_{max}, $M^{-1}\,cm^{-1}$)): 344(22,540), 242(48,763). ^{1}H NMR (DMSO-d_6, ppm): 11.56, 10.83 (s, 1H each, —OH), 8.31(s, 1H, —CH=N^1), 8.44 (s, 1H, —CH=N^2), 7.54, 7.38, 7.25, 6.90(m, 1H each, Aromatic).

2.3.2. Bis(5-chloro-salicylaldehyde) S-methylisothiosemicarbazone (H_2L^2)

H_2L^2 was prepared from the reaction of S-methylthiosemicarbazide (1.165 g, 5 mmol) with 5-chlorosalicylaldehyde (1.556 g, 10 mmol) Yield: 73%. Color: Yellow solid. M.p: 147 °C. Anal. Calc (found). ($C_{16}H_{13}N_3O_2SCl_2$): C, 50.27(50.12); H, 3.43(3.15); N, 10.99(10.74); S, 8.39(8.14). IR (KBr, cm^{-1}): 3270(b) ν(O—H^1); 3283 ν(O—H^2); 1660(s) ν(C=N^1); 1681(s) ν(C=N^2); 1377 ν (C—O). UV–Vis (λ_{Max}, nm (ε_{max}, $M^{-1}\,cm^{-1}$)): 334(22,797), 263(51,564). ^{1}H NMR (DMSO-d_6, ppm): 11.63, 10.75 (s, 2H each, —OH), 8.32 (s, 1H, —CH=N^1), 8.42 (s, 1H, —CH=N^2), 7.78, 7.52, 7.22, 6.88 (m, 1H each, Aromatic).